

Pembrolizumab with low-dose carboplatin for recurrent platinum-resistant ovarian, fallopian tube, and primary peritoneal cancer: survival and immune correlates

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ABSTRACT

Background Anti-programmed death 1 (PD1)/programmed cell death ligand 1 (PD-L1) therapies have shown modest activity as monotherapy in recurrent ovarian cancer. Platinum chemotherapies induce T-cell proliferation and enhance tumor recognition. We assessed activity and safety of pembrolizumab with carboplatin in recurrent platinum-resistant ovarian cancer.

Patients and methods This phase I/II, single-arm clinical trial studied concurrent carboplatin and pembrolizumab in recurrent platinum-resistant ovarian, fallopian tube, and primary peritoneal cancer. Primary platinum refractory patients were excluded. Patients were treated after progression on subsequent non-platinum systemic therapy after becoming platinum resistant or refractory. Pembrolizumab 200 mg was given on day 1 and carboplatin area under the curve 2 on days 8 and 15 of a 3-week cycle until progression. Imaging was assessed by blinded independent review. PD-L1 expression was assessed by immunohistochemistry. Flow cytometry on peripheral blood mononuclear cells was performed for CD3, CD4, CD8, PD1, CTLA4 and Ki67.

Results The most common treatment-related adverse events were lymphopenia (18%) and anemia (9%) with most being grade 1 or 2 (93%). Of 29 patients treated, 23 patients were evaluable for best objective response: 10.3% (95% CI 2.2 to 27.4) had partial response (PR), 51.7% (95% CI 32.5 to 70.6) had stable disease (SD). 56.5% of patients had decreases in target lesions from baseline. All PD-L1-positive patients achieved PR (3/7, 42.8%) or SD (4/7, 57.2%). Median progression-free survival was 4.63 months (95% CI 4.3 to 4.96). Median OS was 11.3 months (95% CI 6.094 to 16.506). Peripheral CD8 +PD1+Ki67+ T cells expanded after 3 (p=0.0015) and 5 (p=0.0023) cycles. CTLA4 +PD1+CD8+ T cells decreased through the course of treatment up to the 12th cycle (p=0.004). When stratified by ratio of peripheral CD8 +PD1+Ki67+ T cells to tumor burden at baseline, patients with a ratio ≥ 0.0375 who had a significantly longer median OS of 18.37 months compared with those

with a ratio < 0.0375 who had a median OS of 8.72 months (p=0.0099). No survival advantage was seen with stratification by tumor burden alone (p=0.24) or by CD8 +PD1+Ki67+ T cells alone (p=0.53).

Conclusions Pembrolizumab with carboplatin was well-tolerated and active in recurrent platinum-resistant ovarian cancer. A ratio of peripheral T-cell exhaustion to radiographic tumor burden may identify patients more likely to benefit from this chemoimmunotherapy.

Trial registration number NCT03029598.

INTRODUCTION

Antibodies targeting the anti-programmed death 1 (PD1)/programmed cell death ligand 1 (PD-L1) pathway have thus far shown only modest activity as monotherapy to treat recurrent advanced ovarian cancer. Pembrolizumab has shown activity in recurrent advanced ovarian cancer with an 8% response rate and a median progression-free survival (PFS) of 2.1 months reported in KEYNOTE-100.¹ Similar response rates and PFS duration are seen with treatment with nivolumab and avelumab in recurrent advanced ovarian cancer.^{2,3} As a result, there is interest in exploring combinations with anti-PD1/PD-L1 agents to improve efficacy for recurrent ovarian cancer.⁴⁻⁷

Cytotoxic chemotherapies have been shown to stimulate the immune system in several ways.⁸ Platinum chemotherapies possess unique immune properties and induce T cell proliferation and cytokine release.^{9,10} Cisplatin and carboplatin promote cytotoxic T cell activity in vitro at concentrations used in vivo.¹¹ Modulation of PD-L1 and PD-L2 has been shown to be mediated through STAT

V.6 and these immune effects have also been demonstrated in mouse models of ovarian cancer.^{12,13} Carboplatin, in particular, induces T cell proliferation in vitro to significantly higher levels compared with other cytotoxic chemotherapies.¹³ We hypothesized these effects could be exploited to synergize with anti-PD1 therapy. We assessed the safety and activity of pembrolizumab with carboplatin in recurrent platinum-resistant ovarian cancer.

Response rates to anti-PD1 therapies in recurrent ovarian cancer have been low, so we also explored whether immune analysis from archival tumor samples or contemporaneous measures of peripheral immune response and tumor burden could identify patients who would benefit from this approach. Higher PD-L1 expression in tumors has correlated with improved response rates to pembrolizumab in ovarian cancer, but without significant improvement in PFS.¹⁴ Tumor-infiltrating lymphocytes at time of diagnosis have been associated with improved survival in ovarian cancer, but their prognostic value may be abrogated after cytotoxic chemotherapy.^{15–17} Peripheral lymphocytes have been associated with survival in ovarian cancer, independent of tumor-infiltrating lymphocytes.¹⁸ Peripheral markers measuring invigoration of exhausted T cells in a ratio with overall tumor burden are associated with prolonged survival with pembrolizumab therapy in other malignancies.¹⁹

PATIENTS AND METHODS

Patient population

After informed consent, patients with recurrent ovarian, fallopian tube, or peritoneal carcinoma were enrolled from May 2017 to October 2018. Key eligibility criteria for this phase I/II single arm trial were platinum-resistant advanced ovarian, fallopian, or peritoneal carcinoma with progression on subsequent non-platinum systemic therapy after becoming platinum resistant or refractory. Primary platinum refractory patients were excluded. Enrolled patients received intravenous infusion of pembrolizumab 200 mg on day 1 followed by intravenous infusion of carboplatin area under the curve 2 on days 8 and 15 of a 3-week cycle for 2 years or until progression or unacceptable toxicity. Tumor imaging was performed prior to cycles 4 and 8, and then every 3 months.

Study design

This phase I/II, single-arm clinical trial was designed to examine the clinical response rate of concurrent platinum and pembrolizumab in patients with recurrent platinum-resistant ovarian, fallopian tube, and primary peritoneal cancer. The primary objectives were to (1) determine the clinical response rate of platinum chemotherapy and pembrolizumab and (2) evaluate whether platinum chemotherapy and pembrolizumab in platinum-resistant ovarian, fallopian tube, and primary peritoneal cancer improves PFS. To determine the clinical response of concurrent pembrolizumab and platinum chemotherapy, serial imaging studies evaluated target lesions and were

assessed by both RECIST V.1.1 and irRECIST V.1.1 by blinded, independent review. Imaging was performed at baseline and prior to cycles 4 and 8 and then prior to every fourth cycle. Target lesion responses were described as (1) complete response, (2) partial response (PR), (3) progressive disease (PD) and (4) stable disease (SD). All adverse events (AEs) were reported according to NCI Common Terminology for Adverse Events V.4.0.

Evaluation of PD-L1 expression on tumor tissue

Archival tumor was obtained for immunohistochemical staining. Tissue slides were shipped to QualTek Molecular Laboratories (Newtown, Pennsylvania, USA) for PD-L1 analysis where they were stained using the PD-L1 IHC 22C3 antibody and expression of PD-L1 was scored by a board-certified pathologist. Each slide was given a modified proportion score (MPS), the overall per cent of positive cells expressing PD-L1. MPS is a variant of a typical proportion score, where mononuclear inflammatory cells that express PD-L1 are counted in conjunction with the tumor cells. Only tumor nests were scored for PD-L1 positivity, so the surrounding stroma PD-L1 staining is excluded from MPS. For this study, MPS $\geq 5\%$ was defined as PD-L1 positive.

Evaluation of immune signatures by flow cytometry

Cryopreserved peripheral blood mononuclear cell (PBMC) samples from baseline and post-treatment (cycles 1–12) were thawed and rested overnight at 37°C. Then cells were stained with a viability dye (eBioscience Fixable Viability Dye eFluor 450) and a master mix of antibodies for surface stains including CD4-BV605 (BioLegend Cat# 317438, RRID:AB_11218995), CD3-PE-Cyanine5.5 (Invitrogen Cat# 35-0036-42, RRID: AB_11220085), CD8a-PE-Cyanine7 (Invitrogen Cat# 25-0088-42, RRID: AB_1659702), PD1-APC (BioLegend Cat# 329908, RRID: AB_940475), CD152-PE-Cy5 (BD Biosciences Cat# 555854, RRID:AB_396177). Cells were next fixed and permeabilized with the eBioscience Foxp3/Transcription Factor Staining Buffer Set (ThermoFisher) and subsequently stained intracellularly with Alexa Fluor 700 antihuman Ki67 antibody (BioLegend Cat# 350530, RRID: AB_2564040). Stained cells were acquired on a BD Canto RUO and analyzed with FlowJo software (FlowJo, RRID:SCR_008520).

Calculation of immune cell:tumor burden ratios

Ki67 +PD1+CD8+ T cell:tumor burden ratios were calculated from flow cytometry performed on PBMC and total RECIST tumor burden measured at enrollment prior to therapy as previously reported. The ratio was calculated as %Ki67 +PD1+CD8+ cells over the RECIST V.1.1 total at baseline.¹⁹

Statistical evaluation

A sample size of 27 patients was calculated to have 80% power to declare statistical significance at level 0.05 when the true response rate of the combinatorial therapy is 50%. Clinical characteristics of the study cohort at the

Table 1 Patient demographics

Characteristic	Median (range)	No.	%
Number of patients treated	–	29	–
Age, years	65 (41–80)	–	–
Disease stage			
IIC	–	1	3.4
III	–	21	72.4
IV	–	7	24.1
Histology			
Serous	–	26	90
Non-serous	–	3	10
Number of previous chemotherapy regimens	4 (2–9)	–	–
Prior bevacizumab therapy	–	21	72.4
Prior PARP inhibitor therapy	–	9	31.0
RECIST V.1.1 tumor burden			
Number of target lesions	3 (1–5)	29	–
Size of longest diameter in largest target lesion (mm)	32.8 (14.8–124.4)	29	–
RECIST V.1.1 total	65.3 (15–198.2)	29	–
BRCA status			
BRCA1	–	4	13.8
BRCA2	–	1	3.4
Negative	–	23	79.3
Unknown	–	1	3.4

time of initial diagnosis were tabulated. Response rate was compared with historical control rates by examining whether 95% CI cover the historical control rate. Time-to-event variables were analyzed using the Kaplan-Meier method. Kaplan-Meier estimates of the survival function with 95% CIs at specific time points (using Greenwood's formula for the SE) were computed. Comparisons for other efficacy end points with the historical control PFS and overall survival (OS) were conducted by examining whether the 95% CI covers the historical control proportions. Statistical analyses were performed using GraphPad Prism V.8.0.2 (GraphPad Prism, RRID:SCR_002798). Correlations were determined by Pearson's *r* coefficient. Repeated measures comparisons were analyzed using the mixed-effects model without the Geisser-Greenhouse correction, with Tukey's multiple comparisons test.

RESULTS

Patient population

A total of 29 patients were enrolled (table 1). The median age of patients was 65 years (range: 41–80). Patients were heavily pretreated with a median of 4 (range: 2–9) prior lines of systemic therapy. Prior bevacizumab and PARP inhibitor therapy was received by 72.4% and 31% of patients, respectively. Most patients had high-grade serous histology and were initially diagnosed at stage 3 or 4.

Safety

Treatment with pembrolizumab and carboplatin was well tolerated overall (table 2). The most common treatment-related (TR) AEs were lymphopenia and anemia. The majority of TR AEs were grade 1 or 2 (94%). Six per cent of AEs were grade 3 with lymphopenia the most common. The two grade 4 AEs were neutropenia and lymphopenia.

Antitumor activity

Of 29 patients treated, 10.3% (95% CI 2.2 to 27.4) had PR, 51.7% (95% CI 32.5 to 70.6) had SD and 17.2% (95% CI 5.8 to 35.8) had PD (figure 1A). One patient achieved disease stability for 45 weeks. There was no difference in best overall rates of response between RECIST and irRECIST methodologies; 56.5% of patients had decreases in RECIST target lesions from baseline (figure 1B). Median PFS was 4.63 months (95% CI 4.3 to 4.96) (figure 2A). Median OS was 11.3 months (95% CI 6.094 to 16.506) (figure 2B). Seven of the 23 evaluable patients (30.4%) had archival tumor with modified per cent scoring ≥ 5 for PD-L1 and all achieved PR (3/7, 42.8%) or SD (4/7, 57.2%) as best objective response. However, there was no significant improvement in PFS (PD-L1 + median 4.63 (95% CI 4.55 to 4.73), PD-L1– median 4.70 (95% CI 0.00 to 9.41), $p=0.87$) or OS (PD-L1 + median 14.37 (95% CI 10.43 to 18.30), PD-L1– median 10.90 (95% CI 7.87 to

Table 2 Adverse events (AEs)

Most common	Possibly, probably, or definitely related		All AEs	
	No.	% of related AEs	No.	% of all AEs
Lymphopenia	97	21.5	100	12.4
Anemia	51	11.3	82	10.2
Hypoalbuminemia	24	5.3	42	5.2
Thrombocytopenia	33	7.3	37	4.6
White blood cell decreased	33	7.3	35	4.3
Hypocalcemia	7	1.6	31	3.8
Hypokalemia	4	0.9	27	3.3
Nausea	21	4.7	26	3.2
Neutropenia	25	5.5	25	3.1
Hypomagnesemia	18	4.0	24	3.0
AE gradings				
1	316	70.0	597	74.0
2	107	24.0	169	21.0
3	26	6.0	40	5.0
4	2	0.0	2	0.0
5	0	0.0	0	0.0

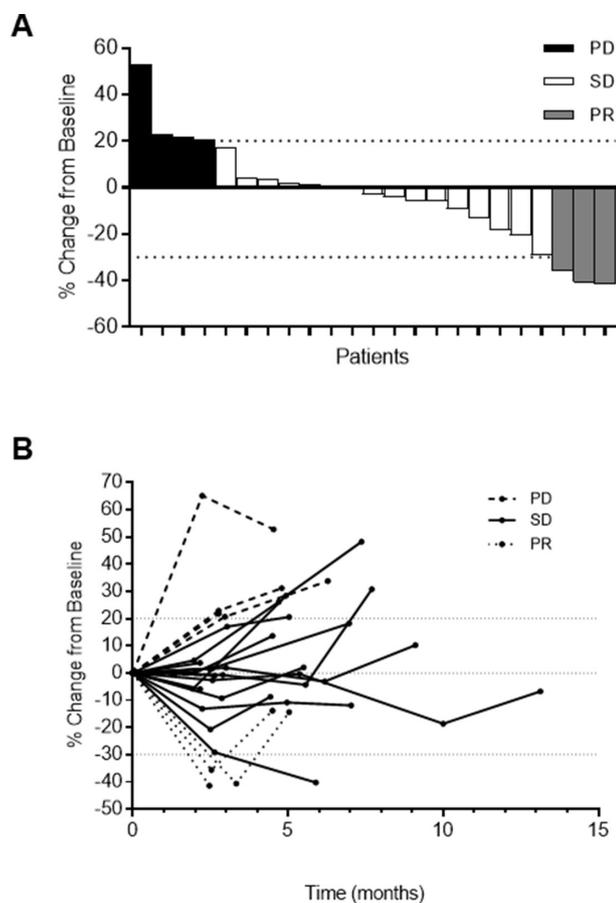


Figure 1 (A) Waterfall plot. Best percentage change in tumor size from baseline by RECIST V.1.1. (B) Spider plot. Percentage change in tumor size over time from baseline by RECIST V.1.1.

13.93), $p=0.96$) in the PD-L1-positive patients compared with PD-L1-negative patients.

Exploratory studies of peripheral immune markers for T cell exhaustion and radiographic tumor burden

Because immunologically relevant circulating T cell populations have been shown to be a potential predictor to PD1 blockade in other malignancies and combinatorial therapy targeting markers in these subpopulations (ie, nivolumab and ipilimumab) have shown activity in ovarian cancer, we performed exploratory flow cytometry on T cells obtained during this study to assess modulation of these markers with pembrolizumab and carboplatin.^{7 19} Peripheral CD8 +PD1+Ki67+ T cells increased significantly from baseline after 3 ($p=0.0015$) and 5 ($p=0.0023$) cycles (figure 3A). We also see significant decreases in the percentages of CTLA4 +PD1+CD8+ T cells through the course of treatment up to the 12th cycle (figure 3B). There was no significant difference in the total peripheral CD8 + population after 3 ($p=0.81$) and 5 cycles ($p=0.21$) (figure 3C).

T-cell exhaustion evidenced by these subpopulations when placed in a ratio with radiographic tumor burden have predicted clinical outcome with anti-PD1 monotherapy in melanoma.¹⁹ We used RECIST V.1.1 criteria for baseline tumor burden as this is the most widely accepted methodology for radiographic tumor quantitation. While we acknowledge this limits the number of baseline target lesions, data warehouse analysis when the RECIST V.1.1 version was implemented showed no loss of information with the move to a reduced lesion number.^{20 21} Stratification by median baseline RECIST tumor burden alone did not yield a significant survival advantage for

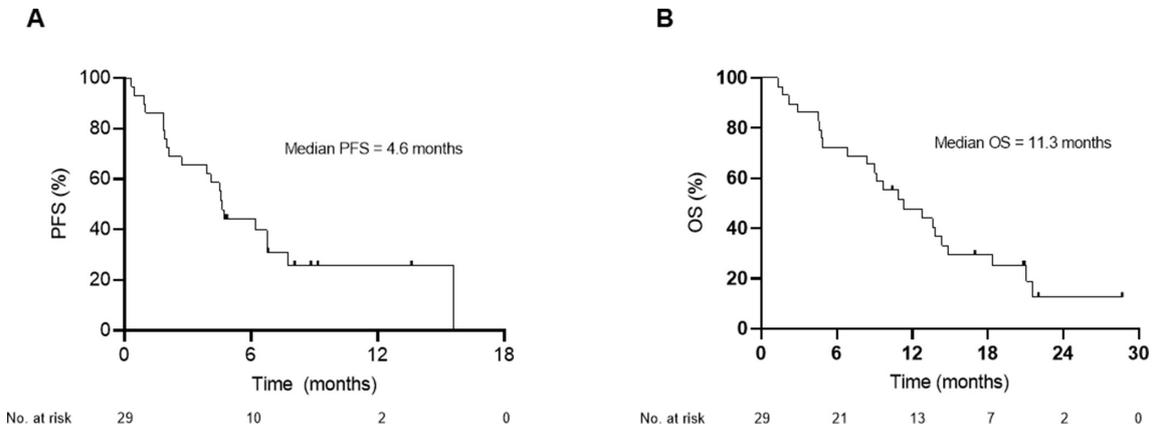


Figure 2 Progression-free survival (PFS) and overall survival (OS). Symbols at censored patients. (A) PFS median (95% CI)=4.633 (4.301 to 4.966), 6-month PFS rate=44.14%. (B) OS median (95% CI)=11.3 (6.094 to 16.506) months.

patients with lower volume disease ($p=0.24$). When patients are stratified by the median ratio of percentage of CD8 +PD1+Ki67+ T cells at baseline to total RECIST tumor burden at baseline, patients with a ratio ≥ 0.0375 had a significantly longer median OS of 18.37 months compared with those with a ratio < 0.0375 who had a median OS of 8.72 months, a 9.65-month OS advantage ($p=0.0099$) (figure 4). No advantage was seen in PFS ($p=0.54$). We tried other cutpoints above and below the median and confirmed that median yields the best contrast between the two groups. Stratification by median CD8 +PD1+Ki67+ T cells at baseline alone also does not yield any significant survival advantage ($p=0.53$).

DISCUSSION

Although the response rate with pembrolizumab and low-dose carboplatin did not differ from monotherapy with anti-PD1/PD-L1 (7.4%–15%) or second-line cytotoxic chemotherapies (11.8%) (figure 5A), median PFS in our trial exceeded what has been reported for single agent cytotoxic chemotherapies without overlap in 95% CIs meeting our defined second primary end point

(figure 5B).^{1-3 14 22} Combinations with anti-PD1/PD-L1 agents to improve efficacy for recurrent ovarian cancer have been explored in other phase II studies, but most are non-randomized and not all have been restricted to platinum-resistant patients.⁴⁻⁷

Low-dose carboplatin with pembrolizumab has favorable tolerability compared with other anti-PD1 combinations in recurrent ovarian cancer.^{4,7} Lower doses of carboplatin used in combination chemotherapy for ovarian cancer have demonstrated improved side-effect profiles, which is of particular importance for regimens contemplated for patients with recurrent ovarian cancer, where PFS must be balanced against toxicities.^{23 24} Both lower doses and metronomic doses of cytotoxic chemotherapies and optimization of the interval between chemotherapy and immunotherapy may also permit modulation of lymphocytes and the immune tumor microenvironment.²⁵

The study has some limitations. It consisted of patients in a single arm and may be subject to selection bias. Patients were more heavily pretreated than similar trials in platinum-resistant ovarian cancer, and although this may represent a group that may be more or less chemoresistant,

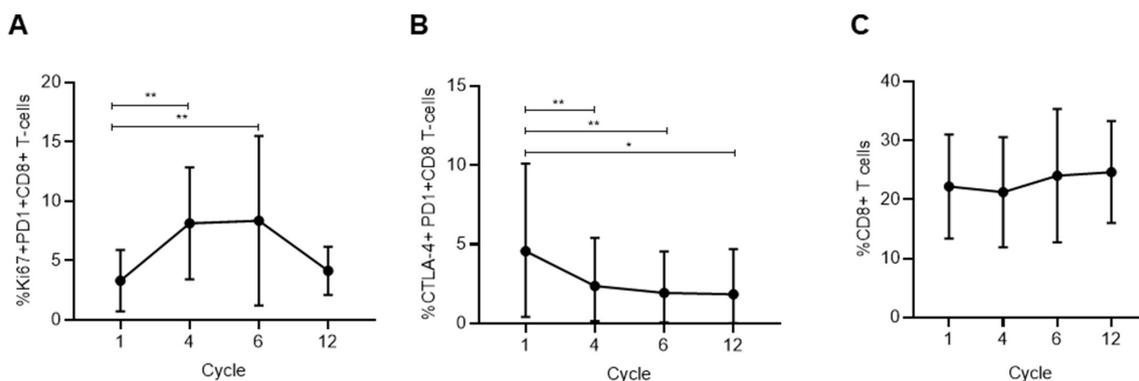


Figure 3 (A) Change in Ki67 +PD1+CD8+ T cells throughout treatment, $p=0.0004$. Cycle 1 vs cycle 4 $p=0.0015$, cycle 1 vs cycle 6 $p=0.0023$. Dots at mean, error bars SD. (B) Change in CTLA-4 +PD1+CD8+ T cells throughout treatment, $p=0.0004$. Dots at mean, error bars SD. Tukey's multiple comparisons test showed the following significant comparisons: cycle 1 vs cycle 4 $p=0.0034$; cycle 1 vs cycle 6 $p=0.0010$; cycle 1 vs cycle 12 $p=0.0336$. (C) Change in CD8 +CD4+ T cells throughout treatment. No significant difference was observed in the total peripheral CD8 + population after 3 cycles ($p=0.81$) and 5 cycles ($p=0.21$). Dots at mean, error bars SD.

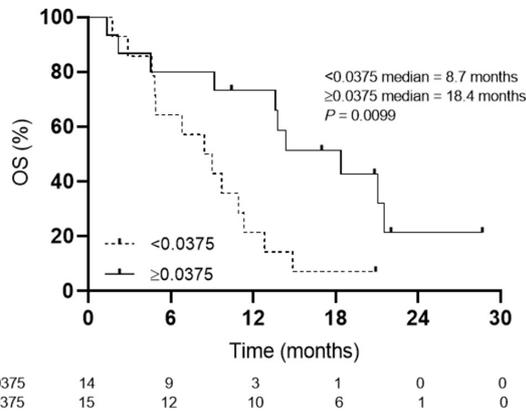


Figure 4 Kaplan-Meier plot of baseline Ki67/tumor burden (TB) ratio stratified by less than and greater than the median ratio (0.0375); <0.0375 median=8.72 months, ≥ 0.0375 median=18.37 months, $p=0.0099$. Symbols at censored patients. OS, overall survival.

it could also represent patients with better prognosis. PD-L1-positive patients showed an improved response rate compared with KEYNOTE-028, which selected this population for treatment with pembrolizumab monotherapy, but the sample size of this study was not powered to detect a difference either in response or survival for this subgroup.¹⁴ Like the AURELIA study, patients refractory to frontline platinum were excluded in this study.²² However, unlike AURELIA, the majority of patients in this study had previously been treated with bevacizumab. While some monotherapy anti-PD1/PD-L1 studies could have included patients refractory to frontline platinum, the contribution appears to be small. In KEYNOTE 100, only 4 patients out of 291 (1.4%) with reported platinum response status were refractory to any line of platinum therapy.²⁶ Responses to platinum chemotherapy after clinical diagnosis of platinum-resistant ovarian cancer are uncommon, but have been reported in retrospective studies. These illustrate that the definition of platinum resistance is imperfect.^{27–29} This study was not designed to establish whether low-dose carboplatin is augmenting response to pembrolizumab or vice versa, but instead sought to identify an additive benefit of combining an immunotherapy with low-dose carboplatin to improve

response rates over monotherapy. Given the imperfect definition of platinum resistance, some cytotoxic contribution from platinum may have occurred in this study. However, the low dose used and high percentage of disease stability favors an immunotherapy effect. Finally, although the markers identified in the exploratory analyses could possess predictive or prognostic value, the design of the study permits us to suggest these as hypothesis generating only.

The OS observed for this study does not differ significantly from what would be expected in a heavily pretreated platinum-resistant ovarian cancer population with standard therapies. The absence of an OS advantage in studies that have shown improved PFS is not uncommon for ovarian cancer and has led to questions regarding the appropriate end points for all ovarian cancer clinical trials.^{22–30} This is particularly relevant for immunotherapies in recurrent ovarian cancer, where there may be improved efficacy with subsequent lines of chemotherapy.³¹

BRCA1 and BRCA2 mutated ovarian cancers have been noted to have a higher neoantigen load, greater numbers of tumor-infiltrating lymphocytes and expression of PD1/PD-L1, which may lead them to be more sensitive to PD1/PD-L1 inhibitors.³² Only five patients in our study had known BRCA1 or BRCA2 mutations so the ability to meaningfully correlate this with response in a study of this size would be limited, but this would be an important line for future inquiry. However, low response rates for immunotherapies in ovarian cancer continue to drive the need for markers to identify patients who would most benefit from these approaches.

Our exploratory analysis of peripheral markers of T cell exhaustion and tumor burden was undertaken after studies identified the significance of these measures in patients with melanoma treated with pembrolizumab monotherapy.¹⁹ The prognostic significance of residual tumor volume in ovarian cancer has long been known.^{33–34} Tumor-infiltrating lymphocytes have been shown to have prognostic significance, but platinum-based chemotherapy can alter the tumor microenvironment along with the prognostic significance of tumor-infiltrating

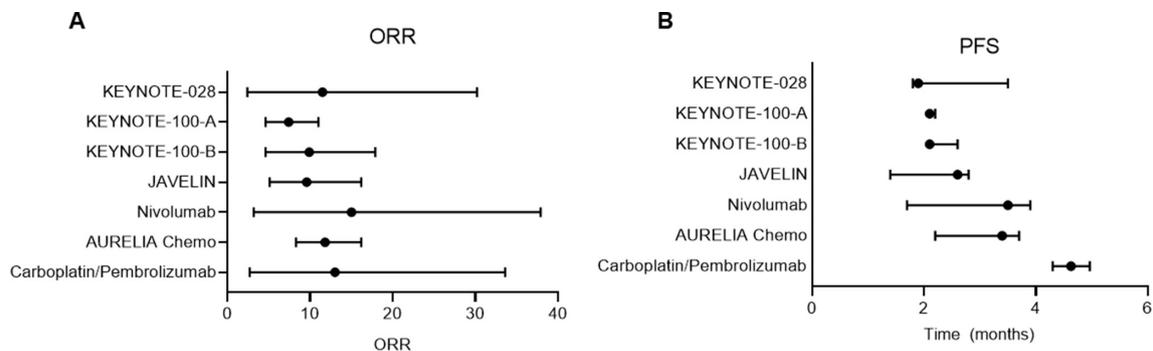


Figure 5 (A) Overall response rate (ORR) comparison. (B) Progression-free survival (PFS) comparison with other monotherapies for platinum-resistant ovarian cancer: KEYNOTE (pembrolizumab),^{1–13} JAVELIN (avelumab),² nivolumab,³ and AURELIA Chemo (paclitaxel, pegylated liposomal doxorubicin, topotecan).²⁰

lymphocytes, so greater tumor burdens may exert immune effects detectable in peripheral T cells from patients with ovarian cancer.^{15–18,35} This analysis generates a hypothesis that such a ratio could represent an accessible marker to select recurrent ovarian cancers for chemoimmunotherapy, since biopsies are not routinely obtained in the recurrent setting, but radiographic imaging is commonly used to confirm recurrences detected by serological tumor markers, such as CA-125.

We see evidence of T cell reinvigoration with expansion of CD8+PD1+Ki67+ T cell populations with carboplatin pembrolizumab therapy without concurrent increase in the total CD8+ population. In recurrent ovarian cancer, early detection of low volume disease is possible with CA-125 surveillance.³⁶ We are studying the potential synergy of carboplatin with pembrolizumab with a modified regimen in patients with ovarian cancer with low volume disease, with biochemical recurrences, to optimize the tumor burden side of this ratio (NCT# 04387227). Overall, these findings suggest that strategies such as using platinum as an immune sensitizer in select patients may improve efficacy of anti-PD1/PD-L1-based therapies, even in cancers considered less immunogenic.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information. Data will be available on ClinicalTrials.gov.

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Supplemental Information

Eligibility and Ineligibility Criteria

Ages Eligible for Study: 18 Years and older

Sexes Eligible for Study: Female

Inclusion Criteria:

Have a diagnosis of ovarian, fallopian tube, or primary peritoneal cancer patients who had a complete response to primary treatment with platinum based chemotherapy, have progressed within 6 months of completing platinum based chemotherapy and have subsequently received at least one, non-platinum-based, therapy

Have relapsed, refractory, or progressive disease following last line of treatment

Have estimated life expectancy of at least 3 months

Be willing and able to provide written informed consent/assent for the trial

Have measurable disease with at least 1 unidimensional lesion based on Response Evaluation Criteria in Solid Tumors (RECIST) 1.1

Have a performance status of 0 or 1 on the Eastern Cooperative Oncology Group (ECOG) performance scale

Within 10 days of treatment initiation: Absolute neutrophil count (ANC) \geq 1,500/mcL

Within 10 days of treatment initiation: Platelets \geq 100,000/mcL

Within 10 days of treatment initiation: Hemoglobin \geq 9 g/dL or \geq 5.6 mmol/L without transfusion or erythropoietin (EPO) dependency (within 7 days of assessment)

Within 10 days of treatment initiation: Serum creatinine \leq 1.5 X upper limit of normal (ULN) OR measured or calculated creatinine clearance (glomerular filtration rate [GFR] can also be used in place of creatinine or creatinine clearance [CrCl]) \geq 60 mL/min for subject with creatinine levels $>$ 1.5 X institutional ULN

Within 10 days of treatment initiation: Serum total bilirubin \leq 1.5 X ULN OR direct bilirubin \leq ULN for subjects with total bilirubin levels $>$ 1.5 ULN

Within 10 days of treatment initiation: Aspartate aminotransferase (AST) (serum glutamic-oxaloacetic transaminase [SGOT]) and alanine aminotransferase (ALT) (serum glutamate pyruvate transaminase [SGPT]) \leq 2.5 X ULN OR \leq 5 X ULN for subjects with liver metastases

Within 10 days of treatment initiation: Albumin \geq 2.5 mg/dL

Within 10 days of treatment initiation: International normalized ratio (INR) or prothrombin time (PT) \leq 1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or partial thromboplastin time (PTT) is within therapeutic range of intended use of anticoagulants

Within 10 days of treatment initiation: Activated partial thromboplastin time (aPTT) \leq 1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication; if the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required

Female subjects of childbearing potential should be willing to use 2 methods of birth control or be surgically sterile, or abstain from heterosexual activity for the course of the study through 120 days after the last dose of study

medication; subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year

Exclusion Criteria:

Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy within 4 weeks of the first dose of treatment

Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment

Short-term administration of systemic steroids (i.e., for allergic reactions or the management of immune related adverse events [irAEs]) is allowed

Has a known history of active TB (Bacillus tuberculosis)

Hypersensitivity to pembrolizumab or any of its excipients

Has had a prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to study day 1 or who has not recovered (i.e., \leq grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier

Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study day 1 or who has not recovered (i.e., \leq grade 1 or at baseline) from adverse events due to a previously administered agent

Note: subjects with \leq grade 2 neuropathy are an exception to this criterion and may qualify for the study

Note: if subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy

Has a known additional malignancy that is progressing or requires active treatment; exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer

Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis; subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment; this exception does not include carcinomatous meningitis which is excluded regardless of clinical stability

Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs); replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment

Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis

Has an active infection requiring systemic therapy

Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator

Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial

Is pregnant or breastfeeding, or expecting to conceive children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment

Clinically significant cardiovascular disease

Known severe hypersensitivity reactions to monoclonal antibodies or carboplatin \geq grade 3, any history of anaphylaxis, or uncontrolled asthma

Has received prior therapy with pembrolizumab

Has a known history of human immunodeficiency virus (HIV) (HIV 1/2 antibodies)

Has known active hepatitis B (e.g., hepatitis B surface antigen [HBsAg] reactive) or hepatitis C (e.g., hepatitis C virus [HCV] ribonucleic acid [RNA] [qualitative] is detected)

Has received a live vaccine within 30 days of planned start of study therapy

Treatment plan, including administration schedule

Experimental: Treatment (pembrolizumab, carboplatin)

Patients receive pembrolizumab IV over 30 minutes on day 1 and carboplatin IV over 30 minutes on days 8 and 15. Courses repeat every 21 days for up to 24 months in the absence of disease progression or unacceptable toxicity.

Measurement of treatment effect including response criteria, definitions of response and survival, and methods of measurement

Progression-free survival (PFS) assessed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [Time Frame: 6 months]

Analyzed using the Kaplan-Meier method. Kaplan-Meier estimates of the survival function with 95% confidence intervals (CIs) at specific time points (using Greenwood's formula for the standard error) were computed.

Comparisons with the historical control PFS will be conducted by examining whether the 95% confidence interval covers the historical control proportions.

1. Response rate (RR) assessed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [Time Frame: 6 months]

The point estimate and the 95% exact CIs will be reported for RR. The comparison with the historical control rate will be conducted by examining whether the 95% confidence interval covers the historical control rate. Additional analyses will be conducted to evaluate in logistic regression models the odds ratio of response rate on predictors such as platinum-free interval, time to progression after previous platinum treatment, number of prior platinum regimens etc.

2. Incidence of adverse events evaluated by National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 [Time Frame: Up to 3.5 years]

The safety population included all patients who received at least one dose of study medication. The descriptions and grading scales found in the revised NCI CTCAE version 4.0 will be utilized for adverse event reporting. The number and the percentage of patients who are removed from the study or altered dose regimen due to adverse effects will be reported.

3. PD-L1 expression of primary tumor blocks assessed by immunohistochemical staining [Time Frame: Up to 3.5 years]

4. Overall survival (OS) [Time Frame: Up to 3.5 years]

Analyzed using the Kaplan-Meier method. Kaplan-Meier estimates of the survival function with 95% CIs at specific time points (using Greenwood's formula for the standard error) were computed. Comparisons with the historical control OS will be conducted by examining whether the 95% confidence interval covers the historical control proportions.

5. Best overall response (BOR) [Time Frame: Up to 3.5 years]

6. Progression-free survival (PFS) assessed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [Time Frame: Up to 3.5 years]

Analyzed using the Kaplan-Meier method. Kaplan-Meier estimates of the survival function with 95% CIs at specific time points (using Greenwood's formula for the standard error) were computed. Comparisons with the historical control PFS will be conducted by examining whether the 95% confidence interval covers the historical control proportions.

7. Immune-related best overall response (BOR) assessed using irRECIST derived from Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [Time Frame: Up to 3.5 years]

8. Immune-related progression-free survival (PFS) irRECIST derived from Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [Time Frame: Up to 3.5 years]

Objectives

Primary Objectives

- (1) **Objective:** To determine the clinical response rate of platinum chemotherapy and MK-3475 in platinum chemotherapy pretreated ovarian, fallopian tube, and primary peritoneal
- (2) **Objective:** To examine whether retreatment with platinum chemotherapy in platinum resistant ovarian, fallopian tube, and primary peritoneal cancers improves progression free survival by concurrent administration of MK-3475.

Secondary Objectives

- (1) **Objective:** To assess the safety and tolerability of concurrent administration of MK-3475 with platinum chemotherapy in patients with platinum resistant recurrent ovarian, fallopian tube, and primary peritoneal cancers.
- (2) **Objective:** To determine the relationship between PD-L1 expression and response to the combination of MK-3475 and platinum
- (3) **Objective:** To assess the overall survival of patients treated with the combination of MK-3475 and platinum

Exploratory Objective

- (1) **Objective:** To explore whether treatment with MK-3475 and platinum alters soluble factors in sera, peripheral immune responses and immune cell profile.

Dose Modification

Dosing interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays).

Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

Subjects with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Subjects will permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug have been previously held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

Subjects will permanently discontinue drug for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

Pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table below.

Table. Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab

General instructions:				
<ol style="list-style-type: none"> 1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks. 2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks. 3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids. 				
Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	1. Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	<ul style="list-style-type: none"> • Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	<ul style="list-style-type: none"> • Monitor participants for signs and symptoms of enterocolitis (i.e., diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (i.e., peritoneal signs and ileus). • Participants with \geq Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal
	Grade 4	Permanently discontinue		

General instructions:				
<ol style="list-style-type: none"> 1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks. 2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks. 3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids. 				
				quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
AST / ALT elevation or Increased bilirubin	Grade 2	Withhold	<ul style="list-style-type: none"> • Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper 	<ul style="list-style-type: none"> • Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 or 4	Permanently discontinue	<ul style="list-style-type: none"> • Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper 	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β -cell failure	Withhold	<ul style="list-style-type: none"> • Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia 	<ul style="list-style-type: none"> • Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	<ul style="list-style-type: none"> • Administer corticosteroids and initiate hormonal replacements as clinically indicated. 	<ul style="list-style-type: none"> • Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue ¹		
Hyperthyroidism	Grade 2	Continue	<ul style="list-style-type: none"> • Treat with non-selective beta-blockers (e.g., propranolol) or thionamides as appropriate 	<ul style="list-style-type: none"> • Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue ¹		
Hypothyroidism	Grade 2-4	Continue	<ul style="list-style-type: none"> • Initiate thyroid replacement hormones (e.g., levothyroxine or liothyronine) per standard of care 	<ul style="list-style-type: none"> • Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	<ul style="list-style-type: none"> • Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper. 	<ul style="list-style-type: none"> • Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		

General instructions:				
<ol style="list-style-type: none"> 1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks. 2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks. 3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids. 				
Myocarditis	Grade 1 or 2	Withhold	<ul style="list-style-type: none"> • Based on severity of AE administer corticosteroids 	<ul style="list-style-type: none"> • Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/persistent Grade 2	Withhold	<ul style="list-style-type: none"> • Based on type and severity of AE administer corticosteroids 	<ul style="list-style-type: none"> • Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Guillain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		
<ol style="list-style-type: none"> 1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. <p>NOTE: For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to \leq Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).</p>				

Carboplatin

Standard dose adjustments for carboplatin during treatment may be made based on changes in hepatic and renal function.

Some of the adverse events expected with carboplatin treatment are listed below.

1. Hematologic: Myelosuppression is the major dose-limiting toxicity
2. Hepatic toxicity: Elevated alkaline phosphatase, total bilirubin, and AST have been observed.
3. Allergic reactions: Hypersensitivity to carboplatin has been reported in 2% of patients receiving the drug. Symptoms include rash, urticaria, erythema, pruritus, and rarely anaphylaxis with bronchospasm and hypotension. The reactions can be successfully managed with standard epinephrine, corticosteroid, and antihistamine therapy.
4. Neurologic: Peripheral neuropathy, ototoxicity, visual disturbances, change in taste, central nervous system symptoms

5. Gastrointestinal: Nausea and vomiting are the most common GI events; both usually resolve within 24 hours and respond to antiemetics. Other GI events include diarrhea, weight loss, constipation, and gastrointestinal pain.

6. Other: Pain and asthenia are the most common miscellaneous adverse events. Alopecia has been reported in 3% of the patients taking carboplatin.

STATISTICAL CONSIDERATION AND ANALYSIS PLAN

Sample size and power

Assume the response rate for the platinum re-treatment therapy in platinum pre-treated ovarian, fallopian tube, and primary peritoneal cancer patients is 23%. One of the primary objectives of this phase I/II trial is to test whether a new combinatory therapy (platinum + anti-PD1 antibody) can achieve a higher response rate (RR). We hypothesize that the true clinically significant response rate for the new therapy is 40–50%. The following table shows the sample size required to have either 80% or 90% power to declare statistical significance at level 0.05 for a true response rate at 40%, 45%, or 50%. A sample size of 27 patients will be required to have 80% power when the true response rate of the new combinatorial therapy is around 50%.

Response Rate of the new Therapy	Power 80%	Power 90%
40%	66	88
45%	41	54
50%	27	37

Furthermore, it is desirable to determine the response rate of the new combinatory therapy with sufficient precision. We can compute the standard error (SE) of the estimated response rates as the measure of precision for a range of true response rates. For the true response rate being in the range of 40%–50%, the SE does not vary much (0.07–0.09), and so there is adequate precision to detect the targeted response rate at 40–50%.

The median progression-free survival time is another efficacy endpoint of interest. Suppose the median progression-free survival time is 17, 20, or 23, and for the target sample size 27, the power to detect a statistically significant improvement in median progression-free survival time assuming accrual time is 1 year and the follow-up time is 1 year is listed in the following table. This table shows that our targeted sample size provides good power to detect 40% or more improvement in median progression-free survival time.

The median PFS in historical control	40% improvement in the new therapy	60% improvement in the new therapy
17 weeks	0.68	0.89
20 weeks	0.66	0.88
23 weeks	0.65	0.89

Statistical analysis plan

Clinical characteristics of the study cohort at the time of initial diagnosis will be tabulated, including age, stage, surgical optimality, and disease status after initial platinum therapy. The clinical characteristics and outcome of platinum-resistant patients will be also tabulated when retreated with the combinatory therapy.

The RR is the primary efficacy variable. The point estimate and the 95% exact confidence intervals will be reported for RR. The comparison with the historical control rate will be conducted by examining whether the 95% confidence interval covers the historical control rate. Additional analyses will be conducted to evaluate in logistic regression models the odds ratio of response rate on predictors such as platinum-free interval, time to progression after previous platinum treatment, number of prior platinum regimens etc. Other efficacy endpoints, which included progression free survival (PFS) and overall survival (OS), were also analyzed. Time-to-event variables were analyzed using the Kaplan-Meier method. Kaplan-Meier estimates of the survival function with 95% CIs at specific time points (using Greenwood's formula for the standard error) were computed. Comparisons with the historical control PFS and OS will be conducted by examining whether the 95% confidence interval covers the historical control proportions.

The safety population included all patients who received at least one dose of study medication. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (<http://ctep.cancer.gov/reporting/ctc.html>)

The number and the percentage of patients who are removed from the study or altered dose regimen due to adverse effects will be reported.